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Gain-of-function screen identifies the role of miR-2 in mitochondrial homeostasis in *Drosophila*

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Abstract: 【Aim】 The homeostasis of mitochondria is maintained by a wide range of molecular processes in eukaryotic cells. Very recently, microRNAs (miRNAs) are emerging as vital players of mitochondrial homeostasis. However, our understanding of how miRNAs regulate mitochondrial homeostasis is still incomplete. Our study aims to examine the roles and mechanisms of miRNAs in mitochondrial homeostasis regulation. 【Methods】 A gain-of-function screen was performed in *Drosophila melanogaster* during which miRNAs were over-expressed in the larval fat body cells and the mitochondrial integrity was monitored. The targets of miRNAs were predicted by bioinformatics methods and RNAi knock-down experiments were performed to examine their effects on mitochondrial morphology. 【Results】 A total of 106 miRNAs were screened in larval fat body of *D. melanogaster*, among which the over-expression of 21 miRNAs resulted in developmental defects of fat body. When over-expressed in the larval fat tissue of *D. melanogaster*, 10 miRNAs led to lethality at the early larval stage, while the other 11 miRNAs led to pupal lethality. Over-expression of miR-2 was found to result in abnormal aggregation of mitochondria in the larval fat body cells of *D. melanogaster*. Sequence analysis revealed that the *Pink*1 gene may be a target of miR-2. The expression level of *Pink*1 gene was shown to be down-regulated by miR-2 over-expression. Genetic interaction experiments demonstrated that over-expression of *Pink*1 was sufficient to rescue the abnormal aggregation of mitochondria caused by miR-2. 【Conclusion】 Our data support the view that miR-2 likely targets *Pink*1 to modulate mitochondrial homeostasis.

Key words: *Drosophila melanogaster*; miRNA; mitochondria; homeostasis; miR-2; Pink1

1 INTRODUCTION

Mitochondria are double-membrane organelles that generate chemical energy in the form of ATP with which cells carry out various vital functions (Mishra and Chan, 2014). During the power generating process, mitochondria interact with multiple metabolism pathways to utilize sugars, fats, and other metabolites as chemical fuels (Spinelli and Haigis, 2018). In addition to energy production, mitochondria perform multifaceted functions such as generation of reactive oxygen species (ROS), regulation of cell signaling and apoptosis (Kasahara and Scorrano, 2014). Therefore, it is not surprising that mitochondrial dysfunction has been implicated in neurodegenerative diseases, metabolic disorders, as well as tumorigenesis (Kasahara and Scorrano, 2014; Mishra and Chan, 2014; Giampazolias and Tait, 2016).

The homeostasis of mitochondria is maintained by balanced actions of mitochondrial biogenesis and degradation (Ploumi *et al.*, 2017). These regulatory machineries are particularly important for energetically demanding tissues such as muscles and nerves. Mitochondrial biogenesis is tightly regulated at the transcriptional level, while clearance of damaged mitochondria is majorly carried out by mitophagy, a selective form of autophagy (Palikaras *et al.*, 2018). Recent discoveries have revealed that the mitochondrially targeted kinase Pink1 plays crucial roles in mitophagy (Ploumi *et al.*, 2017; Palikaras *et al.*, 2018). In healthy mitochondria, Pink1 is cleaved by several proteases at the inner membrane and subsequently degraded by the ubiquitin-proteasome system (Clark *et al.*, 2006). Mitochondrial damages lead to stabilization of Pink1

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on the outer membrane, where it phosphorylates Parkin (Park) to stimulate its ubiquitin ligase activity (Park *et al.*, 2006). Activated Park protein ubiquitinates several outer membrane components and facilitates mitophagy to remove the unhealthy organelle (Yang *et al.*, 2006). Loss of function mutations of *Pink*1 and *Park* are strongly associated with familial forms of Parkinson's disease, illuminating the importance of mitochondrial homeostasis in the health of the nervous system (Mishra and Chan, 2014; Spinelli and Haigis, 2018).

MicroRNAs (miRNAs) are small non-coding RNA molecules comprising of 18 – 25 nucleotides that can cause degradation or translational inhibition of target genes by binding to mRNAs, usually at the 3′-untranslated region (UTR) (Vendramin *et al.*, 2017). Based on the mode of action, each miRNA is estimated to regulate tens of target genes while each gene can be regulated by multiple miRNAs (Geiger and Dalgaard, 2017). Most miRNAs did not show strong phenotypes when loss-of-function mutants were analyzed, but over-expression of miRNAs normally gives rise to observable effects (Chen *et al.*, 2014). Ample evidence revealed that miRNAs regulate mitochondrial function by modulating expression of mitochondrial proteins encoded by nuclear genes (Tomasetti *et al.*, 2014). As of the *Pink*1 gene, more than 20 miRNAs are predicted to bind at the 3′UTR region but only two of them have been experimentally validated (Wang *et al.*, 2015; Kim *et al.*, 2016). Clearly, our understanding of how miRNAs regulate mitochondrial function is incomplete.

Composed by a single layer of large polyploid cells, the *Drosophila* larval fat body is recognized as a great model for studying cellular organelles. A transgenic fly line that marks mitochondria with the yellow fluorescent protein (mtYFP) has been successfully used to visualize mitochondria *in vivo* (LaJeunesse *et al.*, 2004). Taking advantage of this genetic tool, we exploited the potential of studying mitochondrial homeostasis in the fat body of fly larvae. The mtYFP reporter was recombined with a *Cg-Gal*4 driver (Hennig *et al.*, 2006), enabling genetic manipulations in the larval fat body cells by the UAS-Gal4 system. The Hedgehog (Hh) signaling pathway inhibits fat body formation in early developmental stages (Pospisilik *et al.*, 2010), and regulates autophagy as well as lipolysis in mature fat body cells (Jimenez-Sanchez *et al.*, 2012; Zhang *et al.*, 2020). Whether the Hh signaling is involved in mitochondrial homeostasis is unknown. The specificity and sensitivity of this system were examined with transgenes targeting the Hedgehog (Hh) signaling pathway as well as essential mitochondrial components.

Using the fat body mitochondrial morphology as readout, we report a gain-of-function screen in *Drosophila melanogaster* that leads to identification of specific miRNAs as modulators of mitochondrial function. We show that miR-2 is capable of regulating the mitochondrial homeostasis in the larval fat body. We further identified *Pink*1 as the potential target regulated by miR-2 *in vivo*. These results provide novel insights into the molecular mechanisms by which miRNAs regulates mitochondrial function and laid foundation for further studies.

2 MATERIALS AND METHODS

2.1 Fly stocks

Adult *D. melanogaster* flies were maintained in standard medium and crosses were performed at 29℃. The RNAi stocks were obtained from the Tsinghua Fly Center (Table 1). Other RNAi stocks used in this study include: *HLHm*3 (TH01967.N), *HLHm*5 (TH01968.N), *HLHmbeta* (TH01969.N), *Kr-h*1 (TH01976.N), *rpr* (TH02185.N), *CG*4911 (TH02589.N), *CG*11665 (TH02820.N), *Cdk*4 (TH04358.N), *Cht*7 (TH04597.N), *os* (THU0981), *awd* (THU1022), *upd*2 (THU1288), *E* (*spl*) (THU2198), *skl* (THU3063), *Rab*5 (THU3215), *Rab*21 (THU3217), *Sos* (THU3521), *Myd*88 (THU3533), *Pink*1 (THU3783), *Sik*3 (THU3935), *upd*3 (THU4873), *HLH*106 (THU5848), *HLH*4 (THU5849), *HLHmgamma* (THU5851), and *cas* (THU5863). The transgenic miRNA lines of *D. melanogaster* (Bejarano *et al.*, 2012) were obtained from Bloomington Drosophila Stock Center. A stock carrying both the *Cg-Gal*4 driver (#7011) and the *sqh-MitoEYFP* (#7194) marker was generated by standard genetic recombination. The miR-2 sponge stock was obtained from Bloomington Drosophila Stock Center (#61367) and UAS-*Pink*1 was a gift from Dr. WANG Tao. The UAS-*Ptc* (Johnson *et al.*, 1995) and UAS-*HhM*1 (Palm *et al.*, 2013) stocks have been described before.

2.2 Screen design and phenotype scoring

To gain a more thorough view of miRNA function in mitochondrial biology, we screened a collection of 106 transgenic miRNA lines (Bejarano *et al.*, 2012). Each transgenic miRNA line was crossed with the *Cg-Gal*4; *sqh-MitoEYFP* stock individually. At least 50 F_1 progenies were examined for developmental defects. The mitochondrial morphology in the fat body of the 3rd instar larvae of

Table 1　Phenotypes of larval development and mitochondrial morphology of *Drosophila melanogaster* after knock-down of mitochondrial components

Target gene	RNAi stock	Developmental defect	Mitochondrial morphology
*CIA*30	TH02569. N	Normal	Aggregation, large puncta
Scox	TH02747. N	Normal	Aggregation, large puncta
ND-18	TH02297. N	Pupal lethal	Aggregation, large puncta
ND-23	THU0519	Pupal lethal	Aggregation, large puncta
ND-42	THU3600	Pupal lethal	Aggregation, large puncta
ND-49	THU0427	Pupal lethal	Aggregation, large puncta
ND-51	THU0510	Pupal lethal	Aggregation, large puncta
ND-51	THU1792	Pupal lethal	Aggregation, large puncta
ND-75	THU1245	Pupal lethal	Aggregation, large puncta
ND-75	THU2797	Pupal lethal	Aggregation, large puncta
*Cox*15	THU1688	Pupal lethal	Aggregation, large puncta
*TMEM*70	TH02367. N	Larval lethal	Clustered
Parkin	THU5207	Normal	Aggregation
Parkin	THU3738	Normal	Aggregation

Table 2　Phenotypes of larval development and mitochondrial morphology of *Drosophila melanogaster* after miRNA over-expression

miRNA	Bloomington stock	Developmental defect	Mitochondrial morphology
mir-124	41126	Larval lethal	Aberrant
mir-9b	41131	Larval lethal	Aberrant
bft/263-a	41133	Larval lethal	Aberrant
mir-9c	41139	Larval lethal	Aberrant
mir-310	41155	Larval lethal	Aberrant
mir-306, mir-79, mir-9b, mir-9c	41156	Larval lethal	Aberrant
mir-34	41158	Larval lethal	Aberrant
mir-318	41161	Larval lethal	Aberrant
mir-274	41172	Larval lethal	Aberrant
mir-310, mir-311, mir-312, mir-313	41135	Larval lethal	Aberrant
mir-9a	41138	Pupal lethal	Aberrant
mir-263b	41146	Pupal lethal	Aberrant
mir-964	41148	Pupal lethal	Aberrant
mir-276b	41162	Pupal lethal	Aberrant
let-7	41171	Pupal lethal	Aberrant
mir-184	41174	Pupal lethal	Aberrant
mir-278	41180	Pupal lethal	Aberrant
mir-375	41182	Pupal lethal	Aberrant
mir-927	41183	Pupal lethal	Aberrant
mir-8	41176	Pupal lethal	Aberrant
mir-958	41222	Pupal lethal	Aberrant
mir-992	41130	Normal	Clustered
mir-2b-1	41128	Normal	Aggregation

D. melanogaster for each cross was examined. Similarly, each transgenic RNAi line was crossed with the *Cg-Gal*4; *sqh-MitoEYFP* stock individually and examined for mitochondrial morphology.

2.3　Fat body preparation and microscopy

The fat body was dissected from the 3rd instar larvae of *D. melanogaster* and mounted in 80% glycerol without fixation, and samples were examined within 4 h after preparation. The fluorescence images were acquired with a Zeiss Axio Imager Z1 microscope equipped with an ApoTome or a Leica SP8 confocal microscope. The figures were assembled in Adobe Photoshop CS5 with minor adjustments (brightness and/or contrast).

2.4　Real-time quantitative RT-PCR

The fat body was dissected from the 3rd instar larvae of *D. melanogaster* and the total RNA was extracted by Trizol (Invitrogen, USA). The cDNA

was prepared by the First-Strand cDNA Synthesis SuperMix for qPCR (TransGen Biotech, China). Real-time quantitative PCR was conducted using qPCR SYBR Green Master Mix (Yeasen Biotech, China) and the ABI QuantStudio 6 Flex System (Thermo Fisher, USA). Reactions were performed in a 20 μL reaction mixture containing 10 μL Master Mix, 50 nmol/L of each primer, 1 μL cDNA sample and nuclease-free water. The PCR protocol used consisted of a 20 s denaturation at 95℃ followed by 20 s annealing at 59℃ and 20 s elongation at 72℃ for 40 cycles. The housekeeping gene *betaTub85D* was used as the internal control. The primer pairs used for qPCR are as follows: Pink1-F: 5′-TCAATCCCAACCCGTCCAAG-3′; Pink1-R: 5′-CCACTGTAGGATCTCCGGACT-3′; Tub85D-F: 5′-CGGTCAATGCGGTAACCAGAT-3′; Tub85D-R: 5′-ACTATCGCCGTAGTACGTTCC-3′. The primer pairs were designed to amplify a 128 bp fragment of *Pink*1 (NCBI reference sequence: NM_001031878.2) and a 94 bp fragment of *betaTub85D* (NCBI reference sequence: NM_079566.4).

3 RESULTS

3.1 Fly larval fat body is feasible for mitochondrial homeostasis study

We found that transgenes that either activate or repress the Hh signaling were insufficient to alter the mitochondrial morphology when driven by *Cg-Gal*4. As in wild type cells (Fig. 1: A), EYFP-Mito was distributed as punctate structures throughout the cytoplasm in Hh signaling defective fat body cells (Fig. 1: B, C). It has been shown that over-expressed Hh proteins could be secreted from the fat body and reach the wing imaginal disc through circulation (Palm *et al.*, 2013). These external Hh proteins compete with endogenous molecules and dampen the signaling activity in the developing wing (Palm *et al.*, 2013). In agreement with previous studies, Hh over-expression in the larval fat body cells by *Cg-Gal*4 resulted in wing defects resembling Hh signaling reduction. The wings of *Cg-Gal*4 > *HhM*1 flies displayed reduced distance between longitudinal vein L3 and L4, as well as disappearance of the anterior cross vein (Fig. 1: D, E), both are classical Hh deficient phenotypes (Johnson *et al.*, 1995). These experiments suggest that the Hh signaling functions specifically during fat body differentiation but is dispensable for mitochondrial homeostasis in the mature tissue.

The sensitivity of the mtYFP reporter was examined after knock-down of essential mitochondrial components by RNAi (Table 1). Disruption of mitochondrial function by reducing the expression of the electron transport chain complex subunits led to significant changes of the mitochondrial morphology. In general, respiratory chain defective mitochondria formed large aggregates, as shown by knock-down of *CIA*30 (Fig. 2: A, B), *Scox* (Fig. 2: C), *ND*-18 (Fig. 2: D), *ND*-49 (Fig. 2: E) and *ND*-5 (Fig. 2: F) by RNAi. Inhibition of *TMEM*70 by RNAi led to clustering of mitochondria (Fig. 2: G). It is

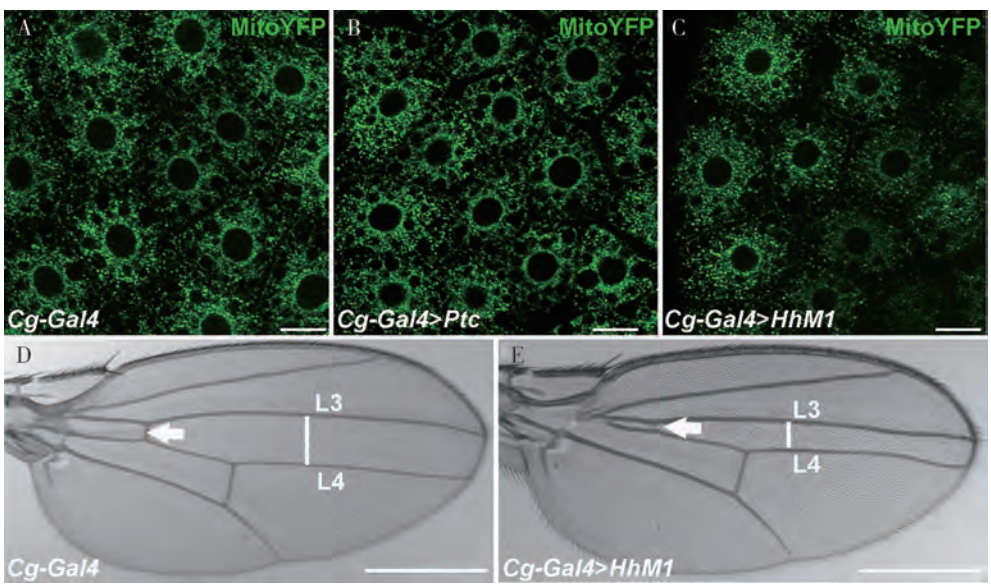

Fig. 1 Hh signaling is dispensable for mitochondrial homeostasis in larval fat body cells of *Drosophila melanogaster*

A – C: Mitochondrial morphology in Hh signaling defective fat body tissues. Wild-type fat body cells are shown as the control (A). The mitochondrial morphology is not altered in cells expressing *Ptc* (B) or *HhM*1 (C). Scale bars = 10 μm. D – E: Adult wing of wild-type (D) and *HhM*1 over-expression (E). In flies with HhM1 ectopically expressed in the fat body tissue, the distance between longitudinal vein L3 and L4 is reduced (white line) and the anterior cross vein is also missing (indicated by white arrows). Scale bars = 0.5 mm.

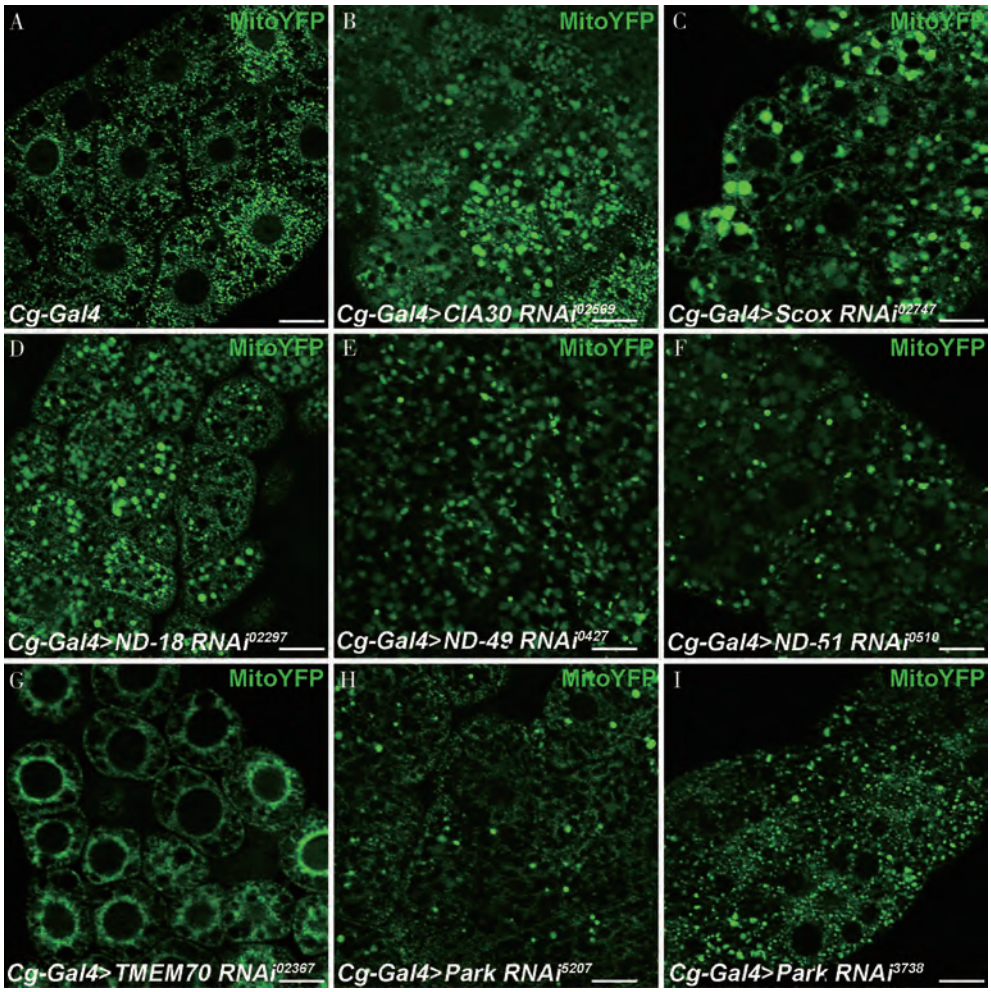

Fig. 2　Knock-down of essential components disturbs mitochondrial morphology in larval fat body cells of *Drosophila melanogaster*
A: Mitochondrial morphology in the wild-type fat body cells is shown as the control; B – F: Knock-down of *CIA*30 (B), *Scox* (C), *ND*-18 (D), *ND*-49 (E) and *ND*-51 (F) by RNAi results in aggregation of mitochondria; G: Knock-down of *TMEM*70 by RNAi leads to clustering of mitochondria; H – I: Inhibition of *Park* expression by two independent RNAi transgenes cause aggregation of mitochondria. Scale bars = 10 μm.

likely that acute malfunction of mitochondria overwhelms the homeostasis machinery, leaving behind many damaged mitochondria to be cleaned up (Zhou *et al.*, 2019). Attenuation of the mitochondrial activity in larval fat body often resulted in systemic growth retardant, and eventually lethality before reaching the adulthood (Table 1). This observation is consistent with the findings that fat body serves as a requisite reservoir for stored lipid during metamorphosis (Li *et al.*, 2019). Interestingly, we found that knocking down the expression of *Park* resulted in mitochondrial aggregation in the larval fat body cells (Fig. 2: H, I). Such phenotype is consistent with *Park* mutants in adult tissues such as muscle and neurons (Clark *et al.*, 2006; Park *et al.*, 2006; Yang *et al.*, 2006), suggesting that mitochondrial dynamics is controlled by the Park-mediated mitophagy pathway in the larval fat tissue (Zhou *et al.*, 2019). The combination of a fat tissue specific Gal4 and mitochondrial fluorescent reporter thus provides an opportunity for systemically studies of mitochondrial homeostasis *in vivo*.

3.2　A genetic screen identifies miRNAs involved in mitochondrial homeostasis

Overall, 83 miRNAs were insufficient to cause neither developmental defects nor visible changes of the mitochondrial morphology. Early larval stage lethality was resulted from over-expression of 10 miRNAs, and lethality at the pupal stage was induced by the over-expression of another 11 miRNAs (Table 2). The 11 miRNAs that caused pupal lethality were found to inhibit the formation of fat tissue, the remaining fat body cells were scattered and the mitochondrial morphology were aberrant (Fig. 3: B, C). For the 10 miRNAs that resulted in early larval stage lethality, very few fat body cells could be recovered but similar mitochondrial morphology defected were recorded (Fig. 3: D). Only two miRNAs were found to regulate

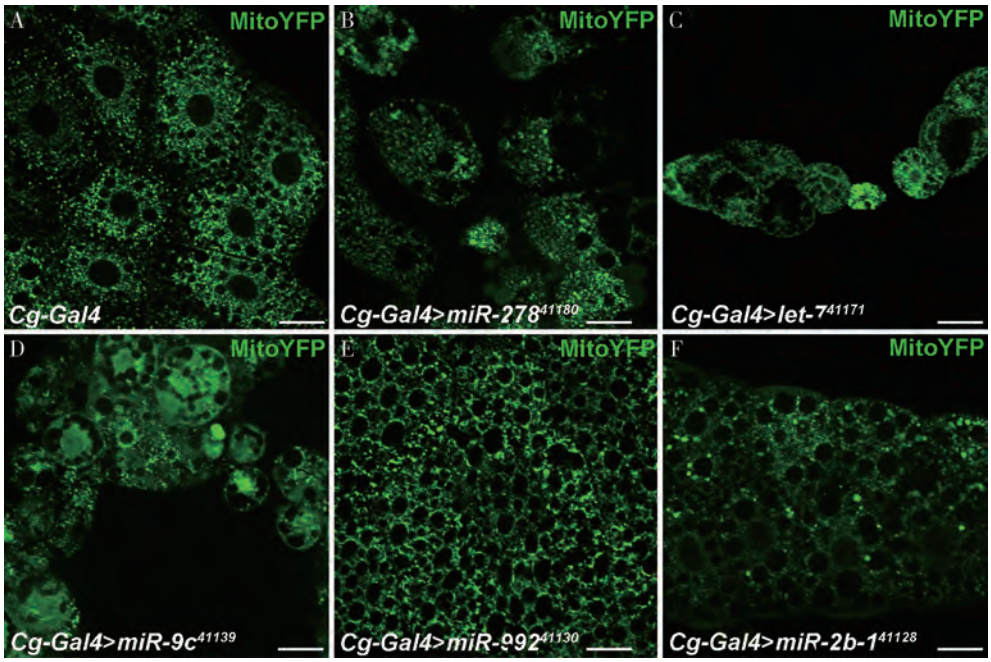

Fig. 3 Gain-of-function screen identifies miRNAs involved in mitochondrial regulation in larval fat body cells of *Drosophila melanogaster*

A: Mitochondrial morphology in the wild-type fat body cells is shown as the control; B – D: Over-expression of miR-278 (B), let-7 (C) and miR-9C (D) interferes with fat tissue formation and disrupts the mitochondrial morphology; E: Over-expression of miR-992 leads to clustering of mitochondria; F: Over-expression of miR-2b-1 results in aggregation of mitochondria. Scale bars = 10 μm.

mitochondrial morphology in the fat tissue without disrupting fly development. We found that in miR-992 over-expressing fat tissue, mitochondria became clustered and formed multiple rings inside one cell (Fig. 3: E). In particular, over-expression of miR-2b-1 led to aggregation of mitochondria, a phenotype resembling that of defective mitophagy caused by *Park* knock-down (Fig. 3: F). Therefore, we performed further genetic studies on the function of miR-2b-1 in mitochondrial homeostasis.

3.3 miR2 likely targets *Pink*1 to regulate mitochondrial health

We first conducted bioinformatics analyses to unveil the potential targets of miR-2b-1 by TargetScan (http: // www. targetscan. org/). We next performed a small-scale RNAi screen targeting 25 potential target genes of miR-2b-1. Interestingly, *Pink*1 was the only target of miR-2b-1 showing mitochondrial phenotype in our RNAi screen. Knock-down of *Pink*1 in the fat body cells led to similar aggregation defects as over-expressing miR-2b-1 (Fig. 4: A, B). We further examined whether *Pink*1 and miR-2b-1 genetically interacts with each other to regulate mitochondrial homeostasis. Knock-down of *Pink*1 together with miR-2b-1 over-expression significantly enhanced the mitochondrial aggregation (Fig. 4: C). *Pink*1 over-expression alone does not cause marked changes in mitochondrial morphology (Fig. 4: D). However, when *Pink*1 was co-expressed with miR-2b-1 in the fat tissue, the mitochondrial aggregates disappeared and the mitochondria looked grossly normal (Fig. 4: E). The rescue efficiency of *Pink*1 is comparable with that of the miR-2 specific sponge (Fig. 4: F) (Loya *et al.*, 2009). The expression level of *Pink*1 gene was down-regulated by miR-2b-1 when examined by real-time quantitative RT-PCR (Fig. 4: G). Taken together, these results suggest that miR-2b-1 likely regulates mitochondrial homeostasis through suppressing *Pink*1 expression.

4 DISCUSSION

During our screen, we found that activation of miR-2b-1 in the larval fat body of *D. melanogaster* led to mitochondrial homeostasis defects without disrupting the overall larval development. miR-2b-1 belongs to the conserved miR-2 family which is involved in developmental events such as suppressing apoptosis (Stark *et al.*, 2003; Leaman *et al.*, 2005), embryonic patterning (Rödel *et al.*, 2013), insect metamorphosis (Song *et al.*, 2019), oogenesis (Lozano *et al.*, 2015) and wing morphogenesis (Ling *et al.*, 2015). To our knowledge, the role of miR-2 in mitochondrial homeostasis has not yet been reported. It has been shown that miR-2 mutant flies displayed slightly higher viability and longer mean median lifespan (Chen *et al.*, 2014). Interestingly, over-expression

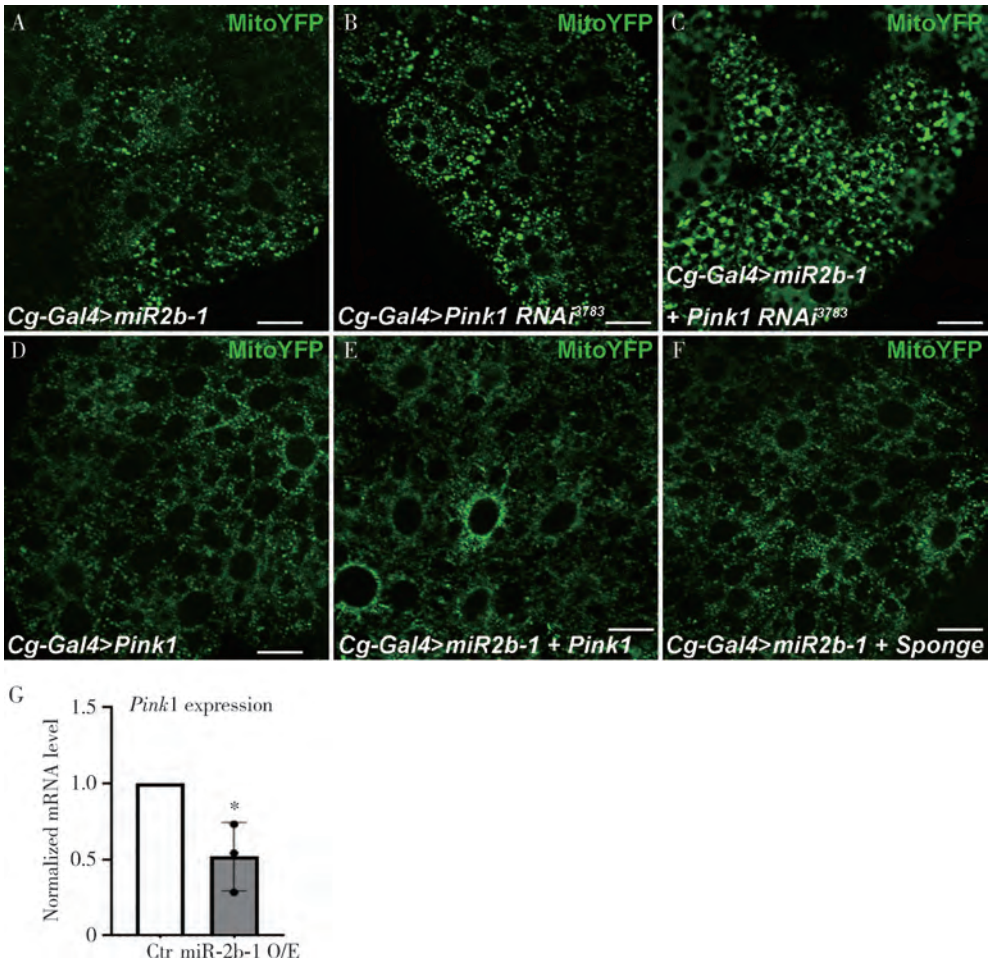

Fig. 4　miR-2b-1 likely regulates *Pink*1 in larval fat body of *Drosophila melanogaster*

A, B: Knock-down of *Pink*1 (A) and over-expression of miR-2b-1 (B) in the fat body cells leads to mitochondrial aggregation. C: Combination of the *Pink*1 RNAi and miR-2b-1 causes severe aggregation of mitochondria. D, E: *Pink*1 over-expression rescues mitochondrial aggregation caused by miR-2b-1. F: The miR-2 sponge is used as the positive control for the rescue experiment. Scale bars = 10 μm. G: The mRNA level of *Pink*1 is down-regulated in miR-2b-1 over-expression fat body cells ($^{*}P < 0.05$, Student's *T*-test).

*of Pink*1 and *Park* is also reported to extend lifespan in fly by reducing proteotoxicity and altering mitochondrial dynamics (Todd and Staveley, 2012; Rana *et al.*, 2013). We reckon that miR-2 might function as a general inhibitor of *Pink*1 in various tissues and developmental stages, therefore removing of miR-2 elevates *Pink*1 expression to protect fly against aging related degeneration. Further studies are needed to demonstrate whether miR-2 directly regulates *Pink*1 expression and to which extent is miR-2 involved in mitochondrial homeostasis.

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References

Bejarano F, Bortolamiol-Becet D, Dai Q, Sun K, Saj A, Chou YT, Raleigh DR, Kim K, Ni JQ, Duan H, Yang JS, Fulga TA, Van Vactor D, Perrimon N, Lai EC, 2012. A genome-wide transgenic resource for conditional expression of *Drosophila* microRNAs. *Development*, 139(15): 2821 – 2831.

Chen YW, Song S, Weng R, Verma P, Kugler JM, Buescher M, Rouam S, Cohen SM, 2014. Systematic study of *Drosophila* microRNA functions using a collection of targeted knockout mutations. *Dev. Cell*, 31(6): 784 – 800.

Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, Yoo SJ, Hay BA, Guo M, 2006. *Drosophila pink*1 is required for mitochondrial function and interacts genetically with *parkin*. *Nature*, 441(7057): 1162 – 1166.

Geiger J, Dalgaard LT, 2017. Interplay of mitochondrial metabolism and microRNAs. *Cell. Mol. Life Sci.*, 74(4): 631 – 646.

Giampazolias E, Tait SW, 2016. Mitochondria and the hallmarks of cancer. *FEBS J.*, 283(5): 803 – 814.

Hennig KM, Colombani J, Neufeld TP, 2006. TOR coordinates bulk and targeted endocytosis in the *Drosophila melanogaster* fat body to regulate cell growth. *J. Cell Biol.*, 173(6): 963 – 974.

Jimenez-Sanchez M, Menzies FM, Chang YY, Simecek N, Neufeld TP, Rubinsztein DC, 2012. The Hedgehog signalling pathway regulates autophagy. *Nat. Commun.*, 39(1): 1200.

Johnson RL, Grenier JK, Scott MP, 1995. Patched overexpression alters wing disc size and pattern: transcriptional and post-transcriptional effects on hedgehog targets. *Development*, 121(12): 4161 – 4170.

Kasahara A, Scorrano L, 2014. Mitochondria: from cell death

executioners to regulators of cell differentiation. *Trends Cell Biol.*, 24(12): 761 – 770.

Kim J, Fiesel FC, Belmonte KC, Hudec R, Wang WX, Kim C, Nelson PT, Springer W, Kim J, 2016. miR-27a and miR-27b regulate autophagic clearance of damaged mitochondria by targeting PTEN-induced putative kinase 1 (PINK1). *Mol. Neurodegener.*, 11(1): 55.

LaJeunesse DR, Buckner SM, Lake J, Na C, Pirt A, Fromson K, 2004. Three new *Drosophila* markers of intracellular membranes. *Biotechniques*, 36(5): 784 – 788, 790.

Leaman D, Chen PY, Fak J, Yalcin A, Pearce M, Unnerstall U, Marks DS, Sander C, Tuschl T, Gaul U, 2005. Antisense-mediated depletion reveals essential and specific functions of microRNAs in *Drosophila* development. *Cell*, 121(7): 1097 – 1108.

Li S, Yu X, Feng Q, 2019. Fat body biology in the last decade. *Annu. Rev. Entomol.*, 64: 315 – 333.

Ling L, Ge X, Li Z, Zeng B, Xu J, Chen X, Shang P, James AA, Huang Y, Tan A, 2015. MiR-2 family targets awd and fng to regulate wing morphogenesis in *Bombyx mori*. *RNA Biol.*, 12(7): 742 – 748.

Loya CM, Lu CS, Van Vactor D, Fulga TA, 2009. Transgenic microRNA inhibition with spatiotemporal specificity in intact organisms. *Nat. Methods*, 6(12): 897 – 903.

Lozano J, Montañez R, Belles X, 2015. MiR-2 family regulates insect metamorphosis by controlling the juvenile hormone signaling pathway. *Proc. Natl. Acad. Sci. USA*, 112(12): 3740 – 3745.

Mishra P, Chan DC, 2014. Mitochondrial dynamics and inheritance during cell division, development and disease. *Nat. Rev. Mol. Cell Biol.*, 15(10): 634 – 646.

Palikaras K, Lionaki E, Tavernarakis N, 2018. Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. *Nat. Cell Biol.*, 20(9): 1013 – 1022.

Palm W, Swierczynska MM, Kumari V, Ehrhart-Bornstein M, Bornstein SR, Eaton S, 2013. Secretion and signaling activities of lipoprotein-associated hedgehog and non-sterol-modified hedgehog in flies and mammals. *PLoS Biol.*, 11(3): e1001505.

Park J, Lee SB, Lee S, Kim Y, Song S, Kim S, Bae E, Kim J, Shong M, Kim JM, Chung J, 2006. Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature*, 441 (7097): 1157 – 1161.

Ploumi C, Daskalaki I, Tavernarakis N, 2017. Mitochondrial biogenesis and clearance: a balancing act. *FEBS J.*, 284(2): 183 – 195.

Pospisilik JA, Schramek D, Schnidar H, Cronin SJ, Nehme NT, Zhang X, Knauf C, Cani PD, Aumayr K, Todoric J, Bayer M, Haschemi A, Puviindran V, Tar K, Orthofer M, Neely GG, Dietzl G, Manoukian A, Funovics M, Prager G, Wagner O, Ferrandon D, Aberger F, Hui CC, Esterbauer H, Penninger JM, 2010. *Drosophila* genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. *Cell*, 140(1): 148 – 160.

Rana A, Rera M, Walker DW, 2013. Parkin overexpression during aging reduces proteotoxicity, alters mitochondrial dynamics, and extends lifespan. *Proc. Natl. Acad. Sci. USA*, 110(21): 8638 – 8643.

Rödel CJ, Gilles AF, Averof M, 2013. MicroRNAs act as cofactors in bicoid-mediated translational repression. *Curr. Biol.*, 23 (16): 1579 – 1584.

Song J, Li W, Zhao H, Zhou S, 2019. Clustered miR-2, miR-13a, miR-13b and miR-71 coordinately target *Notch* gene to regulate oogenesis of the migratory locust *Locusta migratoria*. *Insect Biochem. Mol. Biol.*, 106: 39 – 46.

Spinelli JB, Haigis MC, 2018. The multifaceted contributions of mitochondria to cellular metabolism. *Nat. Cell Biol.*, 20 (7): 745 – 754.

Stark A, Brennecke J, Russell RB, Cohen SM, 2003. Identification of *Drosophila* microRNA targets. *PLoS Biol.*, 1(3): E60.

Todd AM, Staveley BE, 2012. Expression of Pink1 with α-synuclein in the dopaminergic neurons of *Drosophila* leads to increases in both lifespan and healthspan. *Genet. Mol. Res.*, 11(2): 1497 – 1502.

Tomasetti M, Neuzil J, Dong L, 2014. MicroRNAs as regulators of mitochondrial function: role in cancer suppression. *Biochim. Biophys. Acta*, 1840(4): 1441 – 1453.

Vendramin R, Marine JC, Leucci E, 2017. Non-coding RNAs: the dark side of nuclear-mitochondrial communication. *EMBO J.*, 36(9): 1123 – 1133.

Wang K, Zhou LY, Wang JX, Wang Y, Sun T, Zhao B, Yang YJ, An T, Long B, Li N, Liu CY, Gong Y, Gao JN, Dong YH, Zhang J, Li PF, 2015. E2F1-dependent miR-421 regulates mitochondrial fragmentation and myocardial infarction by targeting Pink1. *Nat. Commun.*, 6: 7619.

Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang JW, Yang L, Beal MF, Vogel H, Lu B, 2006. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of *Drosophila* Pink1 is rescued by Parkin. *Proc. Natl. Acad. Sci. USA*, 103(28): 10793 – 10798.

Zhang J, Liu Y, Jiang K, Jia J, 2020. Hedgehog signaling promotes lipolysis in adipose tissue through directly regulating Bmm/ATGL lipase. *Dev. Biol.*, 457(1): 128 – 139.

Zhou J, Xu L, Duan X, Liu W, Zhao X, Wang X, Shang W, Fang X, Yang H, Jia L, Bai J, Zhao J, Wang L, Tong C, 2019. Large-scale RNAi screen identified Dhpr as a regulator of mitochondrial morphology and tissue homeostasis. *Sci. Adv.*, 5(9): eaax0365.

过表达筛选发现果蝇 miR-2 参与调控线粒体稳态

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摘要：【目的】真核细胞内线粒体稳态由多条分子通路共同调控，其中 miRNA 的重要性在最近日益凸显。但是对 miRNA 如何调控线粒体稳态仍缺乏全面准确的认识。本研究旨在探究 miRNA 对线粒体稳态的调控作用及机制。【方法】在黑腹果蝇 *Drosophila melanogaster* 幼虫脂肪体细胞中对 miRNA 进行过表达筛选，监测线粒体形态变化。通过生物信息学方法预测参与调控线粒体稳态的 miRNA 下游靶标，并通过 RNAi 敲低实验检验靶标基因对线粒体形态的影响。【结果】在黑腹果蝇幼虫脂肪体中监测了过表达 106 个 miRNA 对线粒体形态及果蝇发育的影响，发现 21 个 miRNA 过表达引起脂肪体发育异常，其中 10 个 miRNA 过表达可以导致幼虫期致死，11 个 miRNA 过表达导致蛹期致死。过表达 miR-2 导致黑腹果蝇脂肪体细胞中线粒体异样聚集。序列分析表明 miR-2 很可能靶向调控 *Pink*1 基因。*Pink*1 基因表达量确实因 miR-2 过表达而下调。遗传互作实验发现过表达 *Pink*1 基因可以拯救过表达 miR-2 引起的线粒体异样聚集。【结论】结果说明 miR-2 很可能通过靶向 *Pink*1 基因参与调控线粒体稳态。

关键词：黑腹果蝇；miRNA；线粒体；稳态；miR-2；Pink1

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